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**Soil respiration: implications of the plant-soil continuum and respiration
chamber collar-insertion depth on measurement and modelling of soil CO₂
efflux rates in three ecosystems**

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Running head: Measuring CO₂ fluxes from soils

Summary

Key uncertainties remain in accurately measuring soil respiration, including how the commonly-used technique of collar insertion affects measured soil and root-derived CO₂ fluxes. We hypothesized that total soil respiration is frequently under-estimated because soil collar insertions sever surface roots, which coupled to the preferential practice of taking daytime measurements, lead to the autotrophic (root-derived) component frequently being missed. We measured root distribution and soil CO₂ efflux in three contrasting ecosystems: a Lodgepole pine (*Pinus contorta*) plantation, an upland heather-dominated peatland and a lowland sheep-grazed grassland, where we combined shallow surface collars with collars at different soil insertion depths for occasional and continuous hourly flux measurements. Collar insertion by only a few centimetres reduced total soil CO₂ efflux in all three ecosystems by an average of 15% but at times up to 30 to 50% and was directly proportional to the quantity of cut fine roots. Most reduction occurred in the shallow-rooted peatland system and least in the deep-rooted grassland. In the forest and grassland, soil temperatures explained most of the deep collar (i.e. largely heterotrophic) variation and did not relate to the root-derived (i.e. largely autotrophic) flux component, whilst the opposite was true for the peatland site. For the forest, the autotrophic flux component peaked at night during moist periods and was drought-limited. Mean flux estimates differed between sampling time and insertion depth. Our results suggest strongly that accurate measurement and modelling of soil respiration explicitly needs to consider collar insertion, the root-derived flux component, with its own temperature sensitivity and potential time-lag effects.

Introduction

Soil carbon (C) is the largest terrestrial organic C stock (Jiang *et al.*, 2005), representing about two-thirds of terrestrial C (Schimel *et al.*, 1994), of which annually about 75 Pg are lost through soil respiration (Schlesinger & Andrews, 2000). Over time, even small changes in soil CO₂ efflux (soil respiration) will potentially have profound feedback implications on rising atmospheric CO₂ concentration (Schlesinger & Andrews, 2000) and global temperatures (Kirschbaum, 2006). Given that total soil respiration is such a large flux in the global C cycle, it is clearly important to provide as accurate an estimate of this flux as possible. However, soil respiration is a complex flux (Qi *et al.*, 2002), combining root-derived (autotrophic) and decomposition (heterotrophic) C fluxes within a plant-soil continuum (Högberg & Read, 2006), and methodologies of how best to measure soil CO₂ efflux are still under debate and development (Pumpanen *et al.*, 2004; Jiang *et al.*, 2005; Kuzyakov, 2006).

In the recent literature there has been a major emphasis on improving process understanding and modelling of soil respiration and its flux components (Hanson *et al.*, 2000; Reichstein *et al.*, 2003; Kuzyakov, 2006; Bahn *et al.*, 2008). A major focus has been to separate autotrophic and heterotrophic fluxes in the field and to assess their different environmental responses (Heinemeyer *et al.*, 2007; Moyano *et al.*, 2007). A large amount of root, mycorrhizal and microbial activity can be found in the top few centimetres of the soil profile as this is generally the most organic-rich and biologically active layer: litter and organic layers are the main site of nutrient recycling (Kutsch *et al.*, 2001). However, a common perception for soil chamber measurements is the need to install soil collars several centimetres into the soil to avoid any CO₂ leakage out of the chamber (Hutchinson & Livingston, 2001); in peatlands insertion depths up to 30 cm are very common. This practice became established mainly because previous techniques had relied on a relatively large CO₂

increase with possible pump and vent related pressure differences increasing the need for a good seal. Interestingly, deep collar insertion has become such a common practice as not to be specifically reported in many recent soil respiration studies, including cut litter and organic layer depths (see Supplementary Table 1). Only a few studies provide detailed information such as Wang *et al.* (2006), viz: “collars were inserted 5 cm from the ground surface into the soil (including an approximate 1-cm litter layer)”. Consequently, a large and unknown proportion of the autotrophic substrate supply to soil respiration is frequently being missed when inserting collars. Only a few studies addressed this issue systematically in forest as well as peatland soils (Wang *et al.*, 2005a; Silvola *et al.*, 1996). Although the findings of these authors show clear correlations between insertion depth, the amount of cut roots and lost soil CO₂ fluxes, these studies suffer from no pre-treatment monitoring and lacked high frequency (hourly) measurements. Further, the Wang *et al.* (2005a) forest study is based on only a single measurement time and the Silvola *et al.* (1996) peatland study lacked progressive insertion depth and root data.

There is a clear mandate for improved soil respiration monitoring so as to understand the global C budget (Lal, 2003) and if soil CO₂ efflux is not measured accurately, process representation in models using gas exchange measurements to predict the long-term dynamics of soil C pools will remain in error (Falge *et al.*, 2001). For example, soil respiration temperature responses based on a predominantly heterotrophic flux component will be different from those which include the potentially less temperature-sensitive root-derived flux components (Heinemeyer *et al.*, 2007). As Davidson & Janssens (2006) note, “extrapolation of decomposition rates into a future warmer world based on observations of current apparent temperature sensitivities is inadequate”.

An assessment of the published literature for forests illustrates the general importance of considering collar insertion in the context of soil respiration measurements (Supplementary Table 1). The mean collar-insertion depth (where available also considering litter and organic layers) from those studies which provide this information for coniferous, mixed and deciduous forests was 3.5, 5.8 and 4.4 cm (mean, approximately = 4.6 cm), respectively. Results of a similar search for tundra/shrubland, northern peatland as well as tropical peatland studies show a mean collar-insertion depth of 7.0, approximately 16.3 and 6.0 cm (mean = approximately 9.8 cm), respectively (supplementary Table 2). Those few grassland studies provided in Subke *et al.* (2006; Supplementary Table 3) show a mean insertion depth of approximately 2.7 cm ($n = 9$). However, whereas some studies clearly state which layers were cut by collar insertion (Buchmann, 2000), others do not (e.g. Kutsch *et al.*, 2001) and it is usually not clear whether litter and surface organic layers were included in the statement ‘collars were inserted into the soil’ where large amounts of fine roots are commonly found (e.g. Widén & Majdi, 2001). Moreover, in more humid regions collars are often inserted through a deep moss layer (often containing a dense root mat), but this is often inadequately addressed and reflected in collar-depth statement such as ‘inserted into the soil’ (Drewitt *et al.*, 2002). Data for key literature taken from the global soil respiration database (Raich & Schlesinger, 1992) (Supplementary Table 4) spans literature from 1964 to 1989, but provides little information on collar-insertion depth (overall mean of approximately 8.1 cm). However, this global estimate of annual CO₂ soil flux, which is mostly based on old methodologies, known to under-estimate fluxes (alkaline absorption; see Janssens & Ceulemans, 1998), has been cited more than 800 times so far.

We performed a series of field experiments applying different depth of collar insertion (a form of trenching allowing an indirect estimation of heterotrophic (R_h) compared with autotrophic (R_a) respiration fluxes) and related monitored fluxes to root distribution data and

basic site environmental information. Further, long-term infrequent manual measurements were supplemented by short periods of hourly automated flux measurements. The aims of this study were to assess i) the effect of collar-depth insertion on total soil CO₂ efflux, ii) the relationship between flux changes and the amount of cut root, iii) the specific collar insertion implications on the estimated autotrophic component and iv) the effect of measurement frequency and time period on mean flux calculations. All aims were addressed in three contrasting ecosystems, a sandy soil under a Lodgepole pine plantation, an upland heather-dominated peatland site and lowland sheep-grazed grassland.

Materials and methods

Site description

Forest site. This was located within Wheldrake Forest, approximately 5 miles south of York, UK (53°54'34''N; 0°59'48''W, UK Grid Ref SE660463, about 20 m above sea level). The site is a approximately 1-hectare 15-year old Lodgepole pine (*Pinus contorta* Douglas ex Loudon) plantation (with scattered silver birch, *Betula pendula* Roth.) without understorey vegetation. The soil type is a well-draining fine sandy Gley Podzol (FAO, 2006) with a superficial organic layer (approximately 3-cm deep O_a mor type humus under a 2-cm litter layer) overlaying a 3 cm deep A_h horizon with a pH (in H₂O) of around 3.5.

Peatland site. This was located at Moor House National Nature Reserve (Bog End) in the Northern Pennines, UK (54°65'N, 2°45'W; UK Grid Ref NY522768, about 564 m above sea level.). The site is a Histosol (FAO, 2006; commonly known as peat, > 150 cm) supporting

vegetation mainly of *Calluna vulgaris* L. (Hull) and *Eriophorum vaginatum* (Honck.) with some *Sphagnum* ssp. dominated moss patches, classified as *Calluna vulgaris*–*Eriophorum* blanket mire, with a pH (in H₂O) of around 4.3 and a high mean annual water table (approximately 5 cm).

Grassland site. This was located near York (Moor Monkton) on Red House Estate in North Yorkshire, UK (54°00'N, 1°11'W; UK Grid Ref SE536563, about 15 m above sea level.). The site is a permanent grassland (< 20 cm tall) site with sheep grazing, the dominant grasses are *Holcus lanatus* L. and *Lolium perenne* L. with some *Ranunculus repens* L. The soil type is a well-draining fine loamy alluvial Gleysol (FAO, 2006) with a pH (in H₂O) of around 6.5 and approximately 40-cm deep anthropedogenic (hortic) A horizon.

Environmental data

The long-term (1961 – 1990) mean annual precipitation for the forest and grassland sites was 627 mm, with a mean annual air temperature (MAT) of approximately 9.0° C whilst the peatland site received considerably more rainfall (approximately 2000 mm) and had a much lower MAT of 6.0° C (UKCIP98 data from Hulme & Jenkins, 1998).

Mean hourly values were logged (DL2e) for soil temperature (ST4; averaged 10 minute readings, n = 3) in the litter layer, at 5 and 10 cm and soil moisture over the top 6 cm in the mineral soil (ThetaProbe ML2x; n = 1; rotated monthly within the plot), photosynthetically active radiation (QS1; PAR; averaged 10 minute readings, n = 3), wind speed and rainfall (RG1; not for grassland) were monitored at each site (all Delta-T Devices, Cambridge, UK). Occasionally, soil moisture over the top 6-cm mineral soil layer was also measured inside and outside the individual soil collar areas. Air temperature and relative

humidity inside each soil chamber were also recorded during each respiration measurement with the in-built soil chamber sensors (see below).

Experimental design

During April to June and September 2006 and May 2008 an experimental plot was established within each of the forest, peatland and grassland sites, each consisted of a fully balanced randomized block design with four replicates per collar depth treatment.

Collar depth

Within each of four blocks a surface collar (not inserted into the soil; 10 cm diameter PVC collars for the forest and grassland and 20 cm diameter for the peatland site; Plumb Centre, Wolseley UK, Ripon, UK) and three (grassland: four) insertion depth collars were put in place (pushed into the soil after careful pre-cutting with a knife to a target depth) on 10 June, 09 October 2006 and 17 May 2008, for the forest, peatland and grassland sites, respectively. Insertion depths were 5.0, 12.0 and 17.0 cm (forest); 5.0, 10.0 and 20.0 cm (peatland); 2.5, 5.0, 10.0 and 20.0 cm (grassland). Within the forest, short-term measurements were taken with the survey chamber (see below) on three consecutive days from 07 June 2006 (with surface collars at all locations to obtain pre-treatment fluxes) and thereafter on five more occasions on the four blocks at all depth treatments until 15 May 2007. Within the peatland, short-term (pre-treatment) measurements were also taken two times before collar insertion on 08 and 19 September 2006 and on three later occasions (29 November, 04 December 2006 and 27 July 2008). Within the grassland site, manual survey measurements were taken three times before collar insertion on 14, 15 and 16 May 2008 (initial clipping on 14 May) and

thereafter on eight further occasions until 10 September 2008. Collar depth treatments were assigned to each of four blocks on the basis of ranked mean pre-treatment fluxes of the individual collar locations: thus similarly small or large flux areas were allocated a full set of treatments across blocks).

Deep collar-depth treatments are a form of trenching (see Subke *et al.*, 2006) and thus allow an approximate estimate of the autotrophic (R_a) and heterotrophic (R_h) flux components (however, over time decomposing roots might cause additional respiration and deeper roots than insertion were not cut, although root biomass was shown to decline exponentially with depth at all three sites it might re-grow over time), where R_a is assumed to be equal to the difference between surface (0 cm) and deepest (17 or 20 cm) collar treatment fluxes and R_h the difference between surface flux and R_a (notably, this ignores any possible root decomposition effects and R_a from deeper soil layers).

Soil CO₂ efflux measurements

We used a closed dynamic soil CO₂ flux system (LI-COR 8100, model: 8100-101 (8100-104 for the grassland site), LI-COR, Lincoln, Nebraska, USA) for measuring soil CO₂ efflux rates ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) in the field. The system was either used attached to a survey chamber (10 cm diameter, volume 0.84 litre) or to long-term chambers (20 cm diameter, volume 4.07 litre). In the latter case up to 16 chambers were linked to the LI-COR gas analyser unit via a custom-built multiplexed gas handler unit (Electronics Workshop, Biology Department, University of York, UK; for the grassland site a LI-COR multiplexer was used), allowing hourly measurement cycles within a 15-m radius. In both cases, corresponding PVC collars were placed permanently onto or inserted into the soil (shallow surface collars were only pressed down on the shoot-free litter-soil layer with stainless steel fixing hooks (35-cm long

and 2.5 mm diameter welding rods) pressed at an approximately 5° angle 30 cm into the soil). In the forest the undecomposed litter layer (O_i) was first removed (no roots present) from all collar positions, and after mixing a sub sample of 15 g fresh weight (FW) litter was returned onto the collar areas. The peatland site simply required pushing the vegetation to the side and placing the collars onto exposed soil (peat)-root mat using the same fixing hooks as described above. The grassland site required initial (14 May 2008) and subsequent monthly clipping of the vegetation and a commercial weed suppressing membrane (water permeable) was placed over the exposed soil area. The experimental area was protected by a 6 x 4 metre fence to exclude sheep. Any CO_2 diffusion leakage from the surface collars was negligible as the CO_2 increase inside the chamber was limited to less than 35 ppm by adjusting the chamber closure period and, in case of larger increases, the flux calculation time (using the LI-COR software) adjusted to within the first 1 to 2 minutes after closure. Although there were no air gaps directly beneath the collars, the peatland had many air gaps within the deeper root-peat layers. Soil respiration flux rates were computed using the LI-8100 file viewer application software, calculated as a linear CO_2 increase using the 1 s readings and a closure time of around 1 – 2 minutes per hour, discarding an initial approximate 15 s mixing period after closure; circulating air flow rate was set at approximately 1.5 litre minute⁻¹.

Diurnal cycling

We tested the effect of sampling time on measured fluxes per depth treatment with a manual survey chamber (10 cm diameter). However, for the forest and grassland sites a continuous (20 cm diameter) system became available to us unexpectedly, and thus collar adapters were made to fit the 20 cm diameter long-term automatic monitoring chambers by attaching an acetate sheets glued between the 10-cm soil collars and the shallow 20 cm diameter PVC

rings. This method allowed continuous sampling of hourly mean fluxes during the period 13 – 30 June, 05 – 20 October 2006 and 16 June – July 2008 in the forest, peatland and grassland, respectively. However, due to a limitation on the number of automatic chambers at the forest and grassland site, only three blocks were monitored and the deepest (17.0 cm) forest insertion treatment could only be monitored from the 19 June onwards (and could not be monitored in the grassland). Unfortunately, because of power limitations and equipment demands we could not monitor at this hourly frequency (addressing diurnal treatment effects) for long periods at all sites but manual survey sampling extended the measurement period.

Morphological analysis of root system

Forest site. Six soil samples (10 cm diameter x 17 cm length using a PVC corer with a single length side cut to enable easy extraction of the soil core) were taken on 13 and 15 July 2006 from the field site and returned to the laboratory for immediate root extraction. Soil cores were each divided into four soil segment sections (0.0 – 0.5, 0.5 – 5.0, 5.0 – 12.0 and 12.0 – 17.0 cm) and all living roots (identified by appearance, flexibility and colour) were separated from the soil matrix by gently washing with deionized water, before determining morphological features and total fresh weight.

Peatland site. Because of the large root biomass and the resulting long extraction times only three cores were used, which were taken on 27 November 2006 using a 6 x 6 cm square peat corer at 10-m distance from the flux measurement site with root extraction the following week after storage at 4° C. Roots were extracted for three soil segments (0.0 – 5.0, 5.0 – 10.0 and 10.0 – 20.0 cm) by gently washing with deionized water and picking out live roots, as described above. For the two top soil segments a large amount of very fine roots was left at

the bottom of the separating tray and one of 12 randomly selected dividing squares of the bottom tray area was picked out for morphological analysis. Measured root data were subsequently scaled up to the entire tray area and, hence, soil sample.

Grassland site. Four soil cores each (2 cm diameter and 20-cm length) were taken on 29 July 2008 at 1 m distance from the treatment area, cut into four soil segment sections (0.0 – 2.5, 2.5 – 5.0, 5.0 – 10.0 and 10.0 – 20.0 cm) and separated following the above procedure. For morphological analysis, total root length (RL, cm), average diameter and length per diameter class (0.0 – 0.1, 0.1 – 0.2, 0.2 – 0.5, 0.5 – 1.0, 1.0 – 2.0, 2.0 – 5.0, 5.0 – 10.0, 10.0 – 50.0 and > 50.0 mm) per core segment (including the sub-sample) was measured from high resolution (> 600 dpi) scanned images using a WinRhizo® scanner and software package (Régent Instruments, Quebec, Canada). Root dry weight (DW) was recorded after oven drying for three days at 65° C until a constant weight. To avoid disturbance of the soil respiration area for estimating the cut root length, we sampled root cores separately away from (but in the proximity of) the actual respiration collars.

Statistical analysis

Statistical analyses were carried out using SPSS (Version 15, SPSS Science, Birmingham, UK) with Kolmogorov-Smirnov and Levene's tests being used to check for normality and homogeneity of variances. All data were normally distributed (sometimes a log transformation was applied) and fulfilled the requirements of an ANOVA. Individual one-way ANOVAs with an LSD *post-hoc* test on collar treatments were carried out for individual survey measurements before continuous-monitoring commenced, in order to determine whether the CO₂ efflux rates at the different collar treatments differed on different

measurement dates. However, in cases where a significant block effect preventing a *post-hoc* analysis, we applied an independent sample *t*-Test between treatment pairs. Repeated-measures ANOVAs were used to determine whether the CO₂ efflux rates in the different collar treatments changed over time for the combined post-treatment fluxes. For this test the assumption of sphericity was always violated but the *F*-ratios and significance values were based on the more conservative Greenhouse-Geisser corrected degrees of freedom. Significant differences in root and time period flux data were based on a one-way ANOVA and two-way ANOVA, respectively, with an LSD *post-hoc* test.

During the continuous measurements, repeated-measures ANOVAs on the daily mean CO₂ efflux rates, were used to determine whether there was a significant effect on the rate of respiration for the different collar treatments. For the diurnal cycle investigations, repeated-measures ANOVAs, with collar treatment as the between-subject factor, were used to determine whether the CO₂ efflux rates changed significantly over the course of a day and whether there were differences between collar treatments. Linear regressions were used to investigate the relationship between the diurnal cycles of soil temperature and the CO₂ efflux rates at the different collar treatments, and for the corresponding autotrophic (R_a) and heterotrophic (R_h) flux components.

Results

Collar-depth effects on soil CO₂ efflux

In all three ecosystems, and in declining order of peatland, forest and grassland, within a few days after collar insertion in the soil, CO₂ efflux declined significantly with increasing collar

depth when compared with the control surface collar fluxes (placed on top of the litter/peat layer). These reductions were generally around 15 % but increased to 30 – 50 % during peak soil respiration times (Figures 1-3 and 4a,b,c), and is believed to equal to the ‘lost’ autotrophic flux (root-derived) component. This reduction, based on the mean values during the entire hourly flux measurement period, was a long-term effect, as shown from the manual surveys (Figures 1 – 3), although manual survey results mostly showed significant differences only between the surface and deepest collar treatments. Further, collar insertion reduced overall absolute flux variability (see Figures. 1, 2, 3 and 4a,b), which was greatest for surface collars, with the exception of the grassland (note: there was no difference in the relative flux variability (STDEV/mean). Notably, in the peatland and grassland, deep collars resulted in considerable water logging within the collars after heavy rain events, creating standing water as collars prevented lateral water flow.

Diurnal cycling of soil CO₂ efflux

Continuous hourly flux monitoring allowed observations of the diurnal cycling of soil CO₂ efflux and the environmental responses of its estimated component fluxes based on the trenching concept. In the forest, surface collars showed clear diurnal cycling with largest surface fluxes during the night (approximately 23:00 – 05:00) in contrast to the deeper collar treatments (see Figure 4a and also inset Figure 1a), this was also observed for the grassland (Figure 4c) during three periods of high respiration activity (i.e. 16, 25 and 30 June). However, in the shallow rooted peatland (Figure 4b), surface fluxes peaked during midday.

Overall, hourly flux ranges and amplitude were reduced considerably by collar insertion at all sites (Figures 1 (inset), 4a-c). In the forest and grassland, the soil temperatures at 10 cm and soil surface correlated closely to measured total fluxes, respectively (data not

shown). In contrast, for the peatland site soil CO₂ efflux closely followed the daytime peak in soil temperature measured at 2.5-cm depth (Figure 4b). Interestingly, the estimated flux components for the three sites behaved differently in respect to temperature and moisture. In the forest and grassland, the largely estimated autotrophic component (i.e. surface – 17 and 10-cm deep collar fluxes, respectively, see Figure 4a, n = 264 forest; 4c, n= 236 grassland), was only marginally ($r^2 = 0.05$ forest; $r^2 = 0.03$ grassland) affected by soil temperature ($y = 0.07x - 0.57$ forest; $y = -0.05x + 3.16$ grassland). Hence, the mainly heterotrophic component (17 or 10 cm deep collar fluxes or difference between total and estimated autotrophic flux) explained the positive response of the total flux ($y = 0.18x - 0.99$ forest; $y = 0.18x + 1.22$ grassland) to changes in soil temperature at 10 cm or surface soil depth ($r^2 = 0.28$; 0.48 , $P < 0.001$ for forest and grassland, respectively). Surprisingly, this was different in the peatland (see Figure 4b; n = 186) where the heterotrophic component did not respond to temperature changes ($y = 0.02x + 0.26$; $r^2 = 0.05$), and thus the temperature response of the total flux at 2.5 cm soil depth ($y = 0.12x - 0.15$; $r^2 = 0.35$; $P < 0.01$) could only be explained by a response of the largely autotrophic component. However, in the forest, soil moisture appeared to be an important variable in controlling the estimated autotrophic flux component; the forest showed a ‘critical threshold’, separating small ($0.2 - 0.4 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) from large ($0.4 - 0.6 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) autotrophic fluxes above 20% moisture (v/v; data not shown). There was little variation in soil moisture in either the grassland or peatland during the hourly monitoring campaigns; they were consistently wet.

Estimates of root morphology and root respiration

Total RL (Figure 5a-c) declined in the order grassland (80.1 km m^{-2}), peatland (31.2 km m^{-2}) and forest (2.0 km m^{-2}) over 20, 20 and 17 cm soil depth, respectively. There was a large dead

root component in the peatland, but this was not quantified. However, root distribution for the three contrasting sites showed a similar pattern of exponentially decreasing RL densities with depth; in particular, fine-root length was most dominant within the top 10 centimetres (Table 1). Critically, at all sites, around 50% of the total measured RL (Figure 5a-c) was found within the top 5 cm of soil (including litter and organic layers).

On the basis of the root analysis and the field flux measurements, this study also provided an indirect estimate of root-derived respiration. For all three sites we found a very close and near exponential positive relationship between the increasing amount of cut-root length with collar depth insertion and the estimated root-derived fluxes (Figures 5a-c; note: the exponents are negative). The (best fit) polynomial regression equations of cut root length (y) and root-derived flux (x) were: forest $y = -0.0637x^2 + 0.3105x$ ($r^2 = 0.99$), peatland: $y = -0.0006x^2 + 0.0359x$ ($r^2 = 0.98$) and grassland: $y = 0.0001x^2 + 0.0167x$ ($r^2 = 0.72$). The estimated mean R_a (\pm SE during the automated hourly flux periods), as estimated by surface minus deepest collar fluxes, was 0.37 ± 0.02 , 0.52 ± 0.02 and $2.32 \pm 0.79 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ for the forest, peatland, and grassland, respectively, equal to 26, 52 and 38 % of the total (surface collar) soil flux, respectively.

Discussion

Although collar insertion is generally considered necessary to prevent CO_2 leakage out of the chamber, this is a concern for systems with either long closure times (and consequently a large inside-to-outside chamber CO_2 gradient) and/or known pressure artifacts for systems with no adequate pressure vent or unbalanced in-outflow air circulation. Surprisingly, as early as 1974, Edwards was using an automated chamber system, which specifically avoided humus

or soil cutting. Latest chamber systems, such as the LI-COR 8100, require only short closure times (an approximately 35 ppm increase is needed) and a special vent design (Xu *et al.*, 2006) prevents such issues. This can be combined with minimal downward pressure from securing steel hooks and the chamber gasket and leakage becomes negligible. In a review by Davidson *et al.* (2002) much longer closure times have been suggested, which may give rise to chamber-CO₂ increases of around 200 ppm (see Figure 1 in Davidson *et al.*, 2002). On windy days in exposed areas shallow collars may result in smaller flux measurements as pressure can force ambient air inside the chamber volume but the use of a putty or sand chamber sealant can be used to provide an additional seal at the soil-collar base. Although leakage cannot be ruled out completely in the current study, the most convincing evidence against this is the consistently larger soil CO₂ efflux rates measured using the surface collars. Furthermore, in all three ecosystems the wind speeds at ground level under the dense vegetation cover were always very small.

Collar depth and corresponding flux changes

To the best of our knowledge, there are only two limited studies, i.e. Wang *et al.* (2005a) and Silvola *et al.* (1996), which specifically address collar insertion effects, in a forest and peatland respectively, with no such consideration for grasslands. Interestingly, when looking through recent soil respiration literature across ecosystems the reported mean collar insertion depths range from 4.6 cm for forests to 7.0 cm for shrublands and up to 16.3 cm for peatlands (Supplementary Tables 1, 2). In grasslands, insertions of about 2 – 3 cm are commonly observed (based on grassland studies given in Subke *et al.* 2006 and two European Science Foundation Summer School 2004/05 unpublished feedback reports). Our study clearly shows that this is probably resulting in a significant under-estimation of the true soil CO₂ efflux,

with a mean reduction of around 15% but errors up to 30 – 50% at peak (hourly data) flux times depending on the ecosystem. These collar insertions have potentially long-lasting impacts and were maintained here across study periods of 11, 21 and 5 months for forest, peatland and grassland, and are typical for other reported studies (see Supplementary Tables). However, the longevity of any such effect will depend on insertion depth and chamber area as well as on root growth in specific ecosystems. Moreover, a particular collar issue in peatlands (and also for clay soils) is the commonly observed reduction in drainage, resulting in considerable long-term surface water build-up within the collar after rain events. Despite these concerns, there is an increasing tendency not to report collar insertion depths, and even where collar depth is given, it is seldom explicitly stated whether this includes the organic or litter layers, making validation or future correction of the reported fluxes impossible (see Supplementary Tables).

Although not our primary aim, our estimate of R_a based on the continuous monitoring periods of deep collar trenching (see exponential root distributions; Figure 5a-c) of 26, 52 and 38 % , corresponding to average days after ‘trenching’ (dat), for coniferous forest (16 dat), peatland (26 dat) and grassland (38 dat), respectively, compares well to the estimated 50% (root-derived) flux reduction measured with forest girdling by Högberg *et al.* (2001) with a 0.5-cm collar insertion (Högberg & Ekblad, 1996) or the meta-analysis results by Subke *et al.* (2006) of 48 and 33% for temperate coniferous forest and grassland, respectively. However, Subke *et al.* (2006) estimate the R_a of peatlands only to be 15% on the basis of eight studies by the same author (although the actual paper states it to be about 40%, considering the active vegetation period and 2-cm collar insertion, similar to our findings). However, considering the collar depth insertions of the references in the Subke *et al.* (2006) meta-analysis studies (see Supplementary Table 3), it is thus important to see our findings in context for interpreting past data (Subke *et al.*, 2006), questioning if previous estimates of R_a might change if collar

insertion would have been considered. We propose that actually some 55 of those studies (based on insertion depths of greater than or equal to 2 cm out of a total 131) will have had potential larger R_a flux contributions (with approximately 50 providing none or inadequate collar-insertion details), if they were corrected for the reduced root-derived R_a fluxes (approximately 50, 75% and 33% for forest, peatland and grassland, respectively). This very approximate estimate is based on a mean collar insertion depth for each ecosystem (of 4.6 cm for forests; 9.8 cm for peatlands and 2.7 cm for grasslands; see introduction) in relation to our observed reduction in R_a estimates (Fig. 5a-c) caused by cut root and mycorrhizal connections, not considered in the meta-analysis.

We investigated three, in terms of organic matter content, soil microbiology and root distribution, contrasting ecosystems. Although collar-depth insertion (trenching) in our study (17 or 20 cm) did not exclude all root-derived fluxes, the estimated R_a decreased at all sites exponentially with increasing collar depth caused by an increasing amount of cut roots (Figure 5a-c) and mycorrhizal hyphae. Furthermore, Heinemeyer *et al.* (2007) at the same forest site found that a 25-cm collar insertion excluded nearly all the estimated autotrophic flux component (compared with a 75-cm insertion) and the peatland site visibly had few living roots beyond the 20-cm cutting depth. Although the grassland study showed a similar but less pronounced flux reduction this showed a dip in the 5 cm depth treatment (Figure 5c), which was caused by one very fine-root rich collar. This collar effect might still cause under-estimation of root respiration, when using flux data with insertion depths of even a few centimetres for estimating root respiration based on regression methods of root biomass vs. soil flux (Wang *et al.*, 2005b), because of the exponential fine root density distribution. However, the estimate of R_a in our study was made mostly during the active vegetation period and thus might be difficult to compare with annual values stated in, for example, Subke *et al.* (2006), although most of those studies are based on routine manual site measurements (see

Supplementary Table 3), and probably under-estimating the ‘true’ site flux (see analysis results in Table 2).

Diurnal cycling

Collar-insertion depth also reduced diurnal flux variability (see Figure 1, 4a-c; note highest SE in the surface collars, particularly during peak surface collar flux periods), indicating large temporal and spatial variability in the contribution of the root-derived component (although the relative variability did not differ, see earlier). Moreover, soil respiration is commonly measured manually at certain (usually the most convenient) times during the day from about 12.00 to 14.00 hours, allowing for travel time and set-up periods. Considering our comparison (Table 2), this might lead to a bias, similar to the observation by Savage & Davidson (2003). Indeed, assuming such a routine manual sampling regime as is commonly done (e.g. Ward *et al.*, 2007) during the continuous flux sampling period, this resulted in a small, but significantly different daily mean flux from continuous monitoring (Table 2) and was greatest during midday for the peatland and grassland but during night-time for the forest. Interestingly, this sampling time effect decreased with collar depth, also reflected in a decline in fine root density (Table 1), apart from the grassland, which showed a steady decline in fine roots throughout 0 – 10 cm, indicating a time and depth shift in the activity of the R_a component. However, at the forest site there was no real difference under limiting soil moisture (<20% v/v), indicating a drought-reduced forest root-mycorrhizal activity as observed by Heinemeyer *et al.* (2007). A similar study addressing sampling time (Xu & Qi, 2001) found a very small diurnal fluctuation but, importantly, soil collars were inserted 4 cm into the soil. Consequently, although it is not affecting individual treatment comparisons, these methodological differences can result in errors in up-scaled C-flux and budget

calculations. Our observed diurnal variation in soil respiration (particularly in the forest) is also relevant to validating ecosystem process models; potential errors could be introduced when night-time eddy covariance measurements of respiration are up-scaled to estimate day-time respiration fluxes and derive gross primary productivity (Falge *et al.*, 2001; Reichstein *et al.*, 2005). However, such time-lags are still very uncertain, particularly in forests, mostly because of age effects and the unknown C pool mixing (fresh with old) in roots before being used for respiration (see Mencuccini & Hölttä, 2009).

Response of CO₂ flux components to individual environmental properties

In the forest and grassland site, the different responses of estimated R_a and R_h fluxes, as determined by collar trenching, to soil temperature suggest that the R_a component seems to be less temperature-dependent than R_h , as was recently shown for the mycorrhizal component (Heinemeyer *et al.*, 2007; Moyano *et al.*, 2007). Furthermore, in the past reported ‘apparent’ Q_{10} temperature sensitivity values for soil respiration with large r^2 values might relate to soil collar insertion, as the predominantly R_h component was measured by eliminating a large proportion of the more variable R_a (note, consideration of the issue of ‘true’ compared with ‘apparent’ temperature sensitivity is beyond the aim of our study but collar effects on such investigations should be considered). However, in the peatland R_a was the more temperature sensitive flux, possibly be caused by a faster C supply to root respiration in short vegetation and the large soil surface root biomass as well as dampened soil temperature changes in the peat profile. Our findings confirm those of Hartley *et al.* (2007) who suggested that the temperature response of soil respiration depends largely on the autotrophic substrate supply. A time series analysis would be a suitable tool to investigate such lag-response (for example considering PAR levels), but our study did not have enough continuous data to enable us to

do this). Furthermore, for the forest site, we could link limited soil moisture to an overall flux reduction of the autotrophic component, which showed a threshold of around 20% volumetric soil moisture during the dry 2006 summer (data not shown), as suggested by other forest studies (Yuste *et al.*, 2007). Thus any environmental response surfaces (e.g. Reichstein *et al.*, 2003; Saiz *et al.*, 2006) should consider any artifacts on measured flux components introduced by the collar design.

Root morphology and corresponding respiration rates

Cutting with conventional collar insertion inevitably cuts through a large proportion of fine roots in the top few centimetres of soil as found in the study by Wang *et al.* (2005a). We observed an exponential decrease of fine root density with depth at all three sites (Table 1), as can be assumed to be the case in most ecosystems (e.g. Jackson *et al.*, 1996). Indeed, some studies (e.g. Widén & Majdi, 2001) report around 50% of all fine roots in the top 5 cm of soil horizons (including litter and organic layers). However, Wang *et al.* (2005a) assumed this relationship to be linear, although their raw data actually suggest a much better exponential fit (see Figure 2 in Wang *et al.*, 2005a). Consequently, in our study about 50% of the estimated mean R_a flux (based on hourly fluxes) was lost when cutting through the O_e and O_a layer or the top root-peat layer. Our mean root-derived respiration estimates of around 0.30 – 0.50 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ are comparable to other biome fine-root respiration values (Bahn *et al.* 2006), although the grassland had the largest values, obtained during a peak activity period, but similar to those of Bahn *et al.* (2008). The largest value in the grassland was observed during greatest flux rates (9 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) after heavy rainfall, causing water logging in deep collars, and thus reduced flux rates (3.88 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$). However, the calculation of the root-derived flux clearly depends on the time frame used for calculating the ‘lost R_a flux’

(maximum was 1.01, 1.12 and 5.27 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ for the forest, peatland and grassland, respectively). In particular, for studies investigating the relative contribution of R_a/R_h our findings are of significant importance. Interestingly, these collar related artifacts have not been considered in the most recent reviews on this topic (Hanson *et al.*, 2000; Pendall *et al.*, 2004; Kuzyakov, 2006; Subke *et al.*, 2006; Bahn *et al.*, 2008).

Implications for measurements, global data and modelling

We propose that soil respiration methodology for all ecosystems should be reconsidered carefully when using chamber-based approaches, particularly avoiding collar soil insertion and long closure times causing changes to the diffusion gradient. However, this will require using state-of-the-art equipment which is not always available. Notably, pump pressure artifacts might not have been detected readily, as in the methodology comparison by Pumpanen *et al.* (2004) or Le Dantec *et al.* (1999). Only a sealed bottom chamber will enable detection of any such issues, as otherwise soil air will equilibrate pressure differences, possibly explaining very large annual fluxes as reported in Kutsch *et al.* (2001). Such an inter-chamber-comparison study still needs to be done.

Our findings suggest that many studies, such as the frequently cited global estimates of annual CO_2 soil flux (Raich & Schlesinger, 1992), have probably under-estimated total soil flux rates by around 10 – 20% (on the basis of collar insertion alone), probably by more in peatlands. Moreover, because of the fine root densities and collar insertion at the soil surface, past attempts to estimate the global autotrophic soil flux component based on literature values (e.g. Subke *et al.*, 2006) also need to be revisited. However, our findings only focused on one of many chamber-based issues and still need to be tested elsewhere. In particular, the relevance of measuring a true and diurnal (autotrophic) soil CO_2 efflux is of crucial

importance for model validation and advancing our process understanding of the soil flux components from diurnal to seasonal scales (Heinemeyer *et al.*, 2007, Sampson *et al.*, 2007). We therefore recommend that future studies concerned with total soil respiration should consider collar issues and sampling time regimes, with an effort to accurately measure total and component soil respiration fluxes. Moreover, to overcome chamber related limitations or artifacts when investigating R_a vs. R_h flux contributions, both stable isotopes (Moyano *et al.* 2009) and/or improved non-intrusive membrane technology (Flechard *et al.*, 2007) might become powerful tools.

Conclusion

Our research on collar insertion depth and soil CO_2 efflux implies that soil respiration has a large root-derived R_a flux component near the soil surface with potentially strong diurnal cycling and unique environmental response, that is different to the heterotrophic component. Past collar based measurements of soil respiration fluxes might have significantly underestimated the autotrophic component of soil CO_2 efflux by cutting through a large part of the autotrophic soil surface flux network. Secondly, infrequent measurements in time can result in significantly different estimates of total ecosystem soil respiration. Moreover, although sampling frequency might not considerably alter the average flux calculation, understanding and modelling component fluxes and their environmental responses requires high temporal resolution monitoring, in particular in systems with a potential for lag-time periods of below-ground photosynthate allocation. Thirdly, collar-insertion depth is generally considered necessary to prevent CO_2 leakage out of the chamber, but such concerns are mostly based on data from systems with particular chamber shortfalls such as long closure times and/or known

pressure artifacts. Finally, collar-insertion depth has a potentially long-lasting effect on measured flux rates and needs to be considered when interpreting past data and planning future studies. This demonstrates the need either to avoid insertion or to measure the amount of cut roots when inserting collars, and for the deployment of less intrusive techniques such as stable isotopes or membrane techniques.

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NB Supplementary material

This manuscript is accompanied by four supplementary tables and one combined reference
list for those tables.

Figure Captions

Figure 1 Mean soil respiration (CO_2 efflux) \pm SE for the different collar depths on eight days during the period 07 June 2006 to 15 May 2007 at the forest site, all measured at 14.00 hours. Collar-depth treatments started on 10 June. Inset shows a sample of the 17-day period of continuous mean hourly flux monitoring (\pm SE; $n = 3$) at the same collars during the period 28 to 30 June 2006 (arrow indicates period of continuous monitoring). Inset note: largest fluxes and variability (SE) occur for the surface collars (Surface) during the night. Asterisks (** $P < 0.01$) indicate significant treatment effects (Surface compared with all other treatments; no other significant differences were observed) of individual one-way ANOVAs ($n = 4$), overall repeated measures ANOVA was significant at the * $P < 0.05$ level for all post-treatment Surface treatments (i.e. excluding 7 to 9 June). There were no significant differences between pre-treatment fluxes.

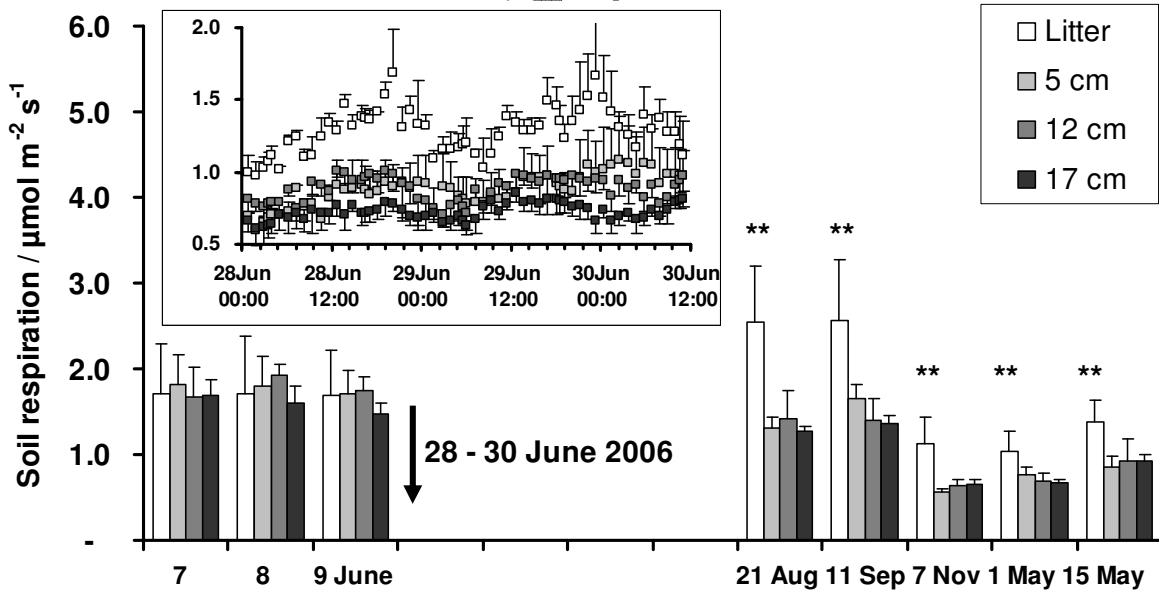


Figure 2 Mean soil respiration (CO_2 efflux) \pm SE for each collar depth treatment on four days during the period September 2007 to July 2008 at the peatland site; the dashed line separates measurements taken before (all surface collars) and after treatment started. Individual one-way ANOVAs ($n = 4$) were significant at the $**P < 0.01$ level for the post-treatment date of 29 November, $*P < 0.05$ for 04 December and $***P < 0.001$ for 27 July; letters indicate *post-hoc* (LSD) test differences between treatments. There were no significant differences between pre-treatment fluxes.

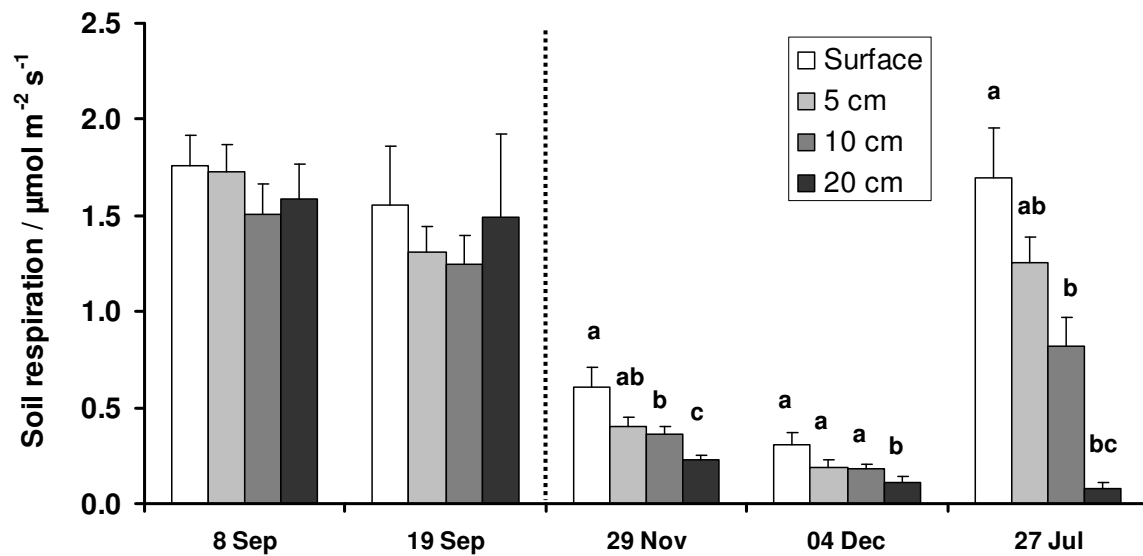


Figure 3 Mean soil respiration (CO_2 efflux) \pm SE for the collar depth treatments on 11 sampling days during the period May to September 2008 at the grassland site; the dashed line separates three measurements taken before (all surface collars) and eight after treatment started. Individual one-way ANOVAs ($n = 4$) were significant at the $*P < 0.05$ level for post-treatment dates 21 May, 05, 10 June, 16 July and 05 August; letters indicate *post-hoc* (LSD) test differences between treatments and stars significance based on independent sample *t*-Tests between Surface and deepest collar treatment only. There were no significant differences between pre-treatment fluxes.

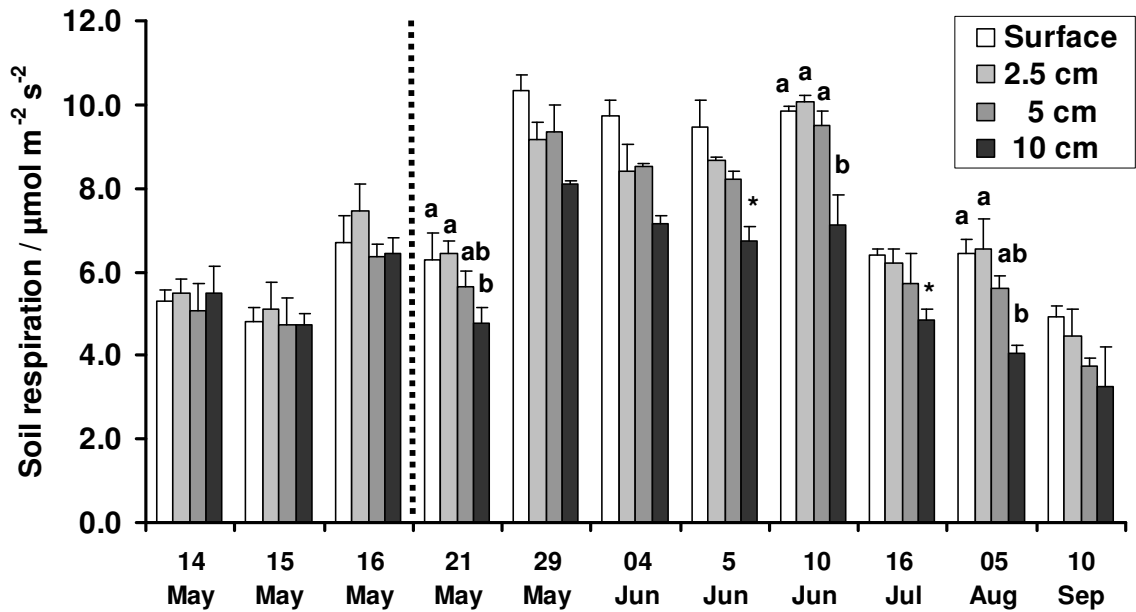
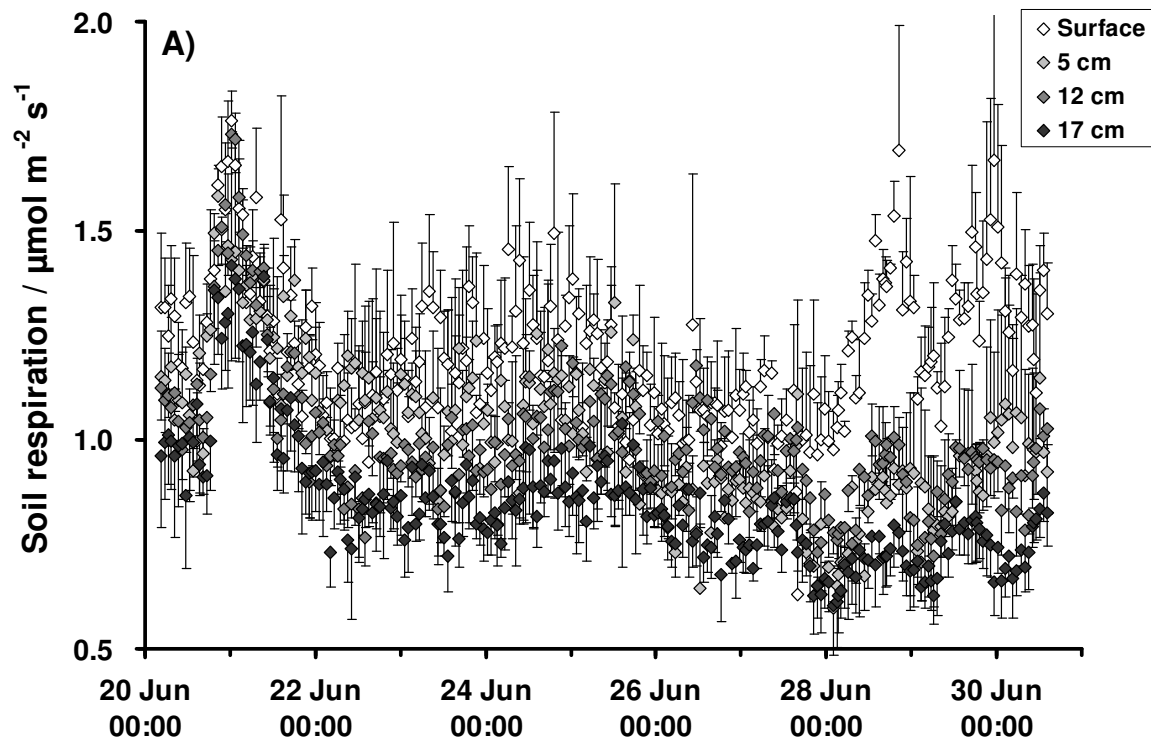
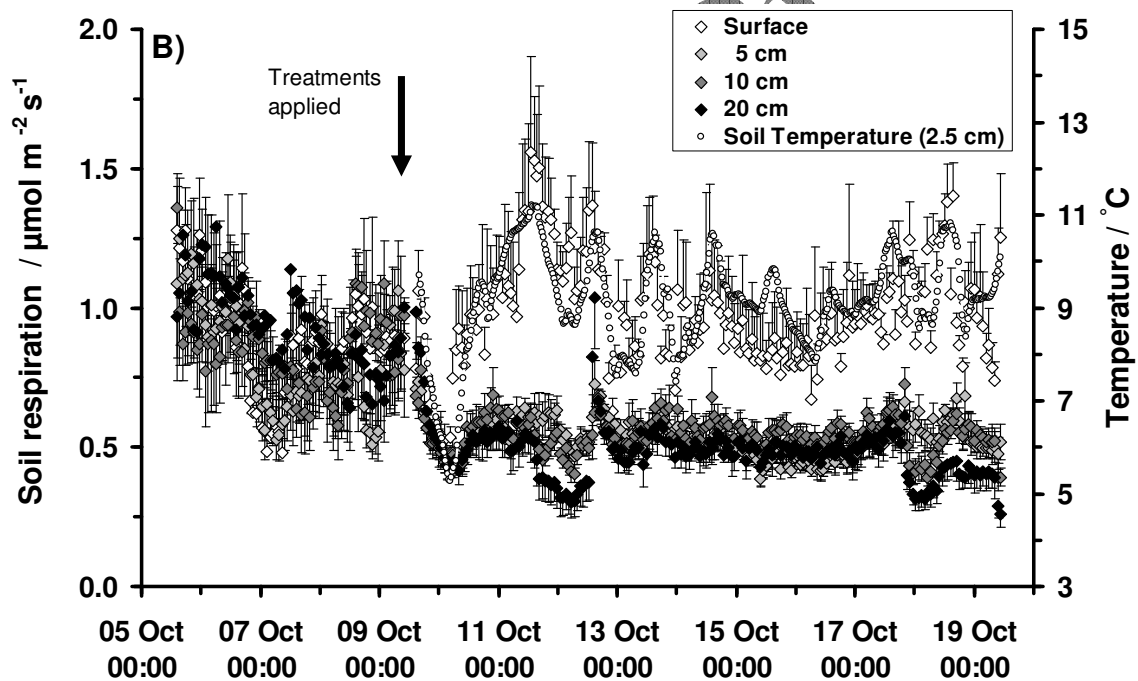


Figure 4 Diurnal and daily variation of continuously measured mean hourly soil respiration (CO₂ efflux) rates \pm SE for the four collar treatments.

A) The forest site. Daily mean fluxes ($n = 12$) of the individual treatment replicates ($n = 3$) during 19 to 30 June and hourly mean fluxes ($n = 63$) of the post-treatment period from 28 to 30 June were significantly different at the $*P < 0.05$ level during this post-treatment period and showed no significant depth \times time interaction. *Post-hoc* differences (Bonferroni's) indicated these differences to be between the Surface and 17 cm treatment.



B) The peatland site. Soil temperature at 2.5-cm depth (open circles) and the onset of treatment (arrow) are also shown. Daily mean fluxes ($n = 10$) of the individual treatment replicates ($n = 4$) between 09 to 19 October and hourly mean fluxes of the post-treatment period from 10 to 14 ($n = 99$) and 15 to 19 ($n = 92$) October were significantly different at the $**P < 0.01$ level during this post-treatment period and showed a weak depth \times time interaction ($*P < 0.05$). *Post-hoc* differences (Bonferroni's) indicated these differences to be between the Surface and all other depth treatment. Note, declining fluxes on 10 October were due to rainfall and a considerable temperature drop event.



C) The grassland site. Daily mean fluxes ($n = 6$) during large flux periods (16, 17, 25, 26, 29, 30 June) for the individual treatment replicates ($n = 3$, but excluding the 5 cm treatment) were significantly different at the $*P < 0.086$ level and showed a weak depth effect ($*P < 0.05$). Hourly mean fluxes ($n = 236$, but excluding the 5 cm treatment) between 13 to 17 June and 25 June to 01 July were significantly different at the $*P < 0.05$ level and showed a weak depth effect ($*P < 0.05$). *Post-hoc* differences (LSD) indicated these differences to be between the Surface and the 10-cm collar depth treatment. Note: large fluxes in the 5-cm treatment reflected a large flux from one collar with a visibly large fine root matt at the surface.

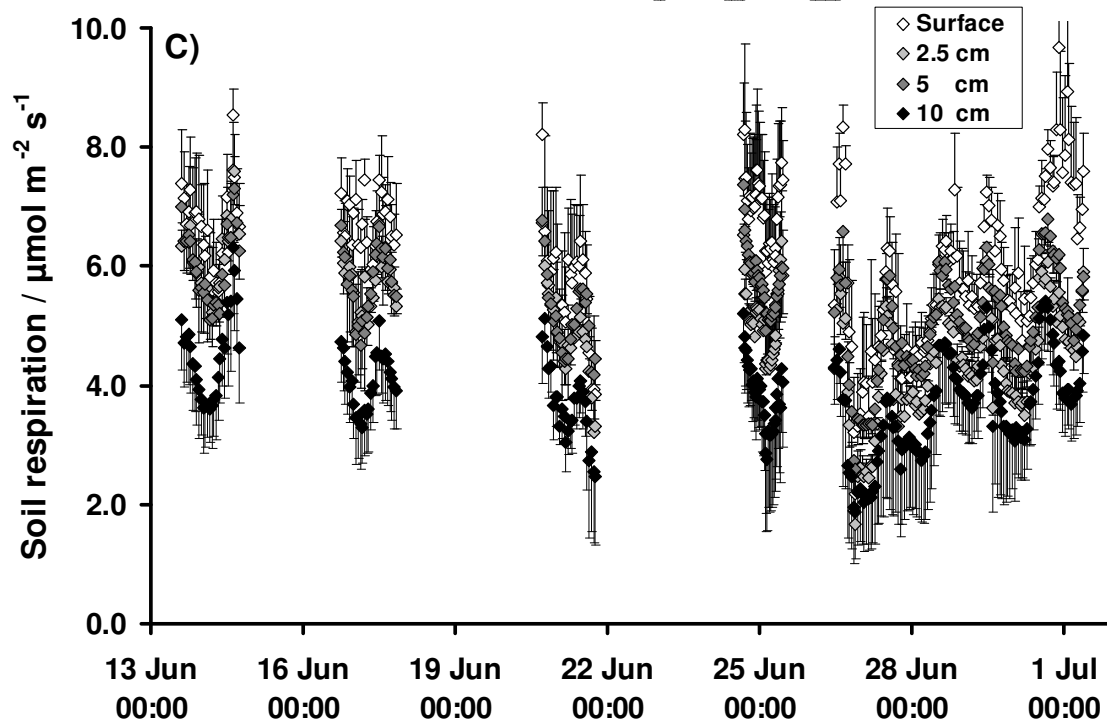
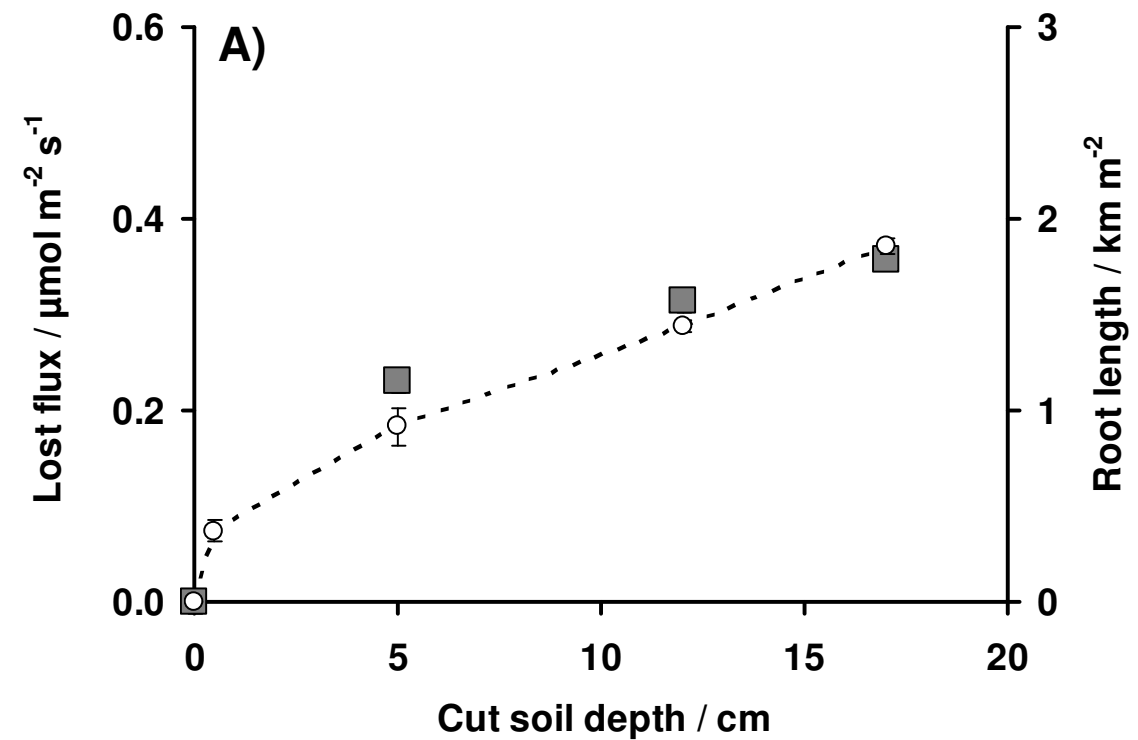
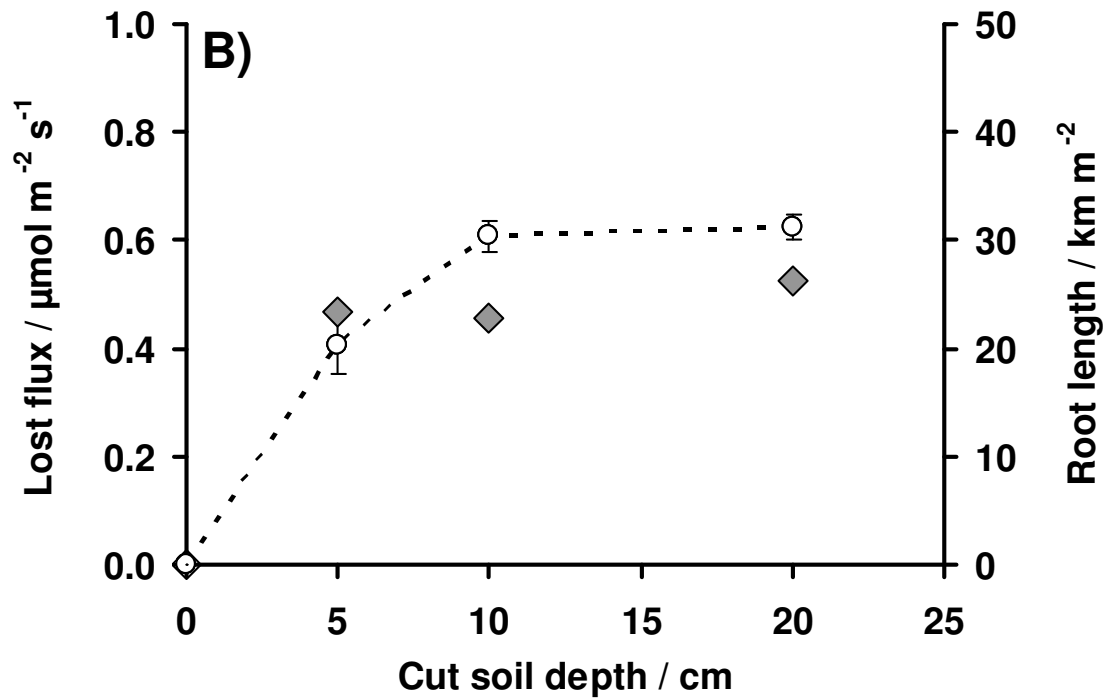


Figure 5 Cumulative root length (mean \pm SE, white circles; dashed line) data (right y-axis) per cut soil depth and the estimated mean \pm SE (less than $0.05 \mu\text{mol m}^{-2} \text{s}^{-1}$) estimated lost root-derived CO_2 fluxes (left y-axis), based on subtraction of Surface flux minus the corresponding collar insertion (cut soil depth; cm) fluxes.

A). The forest site. Average hourly lost soil efflux rates from the four replicated collar depths treatment blocks (grey squares) taken during 19 - 30 June 2006 ($n = 264$) at Wheldrake Forest (root length $n = 6$).



B) The peatland site. Average hourly lost soil efflux rates from the four replicated collar depths treatment blocks (grey diamonds) during the continuous post-treatment monitoring period (n = 191) during 10 – 19 October 2006 at Moor House (root length n = 3).



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870 C) The grassland site. Average hourly lost soil efflux rates from the three replicated collar
 871 depths treatment blocks (grey triangles) during the continuous post-treatment monitoring
 872 period (n = 237) during 13 June – 01 July 2008 at Red House estate (root length n = 4).
 873

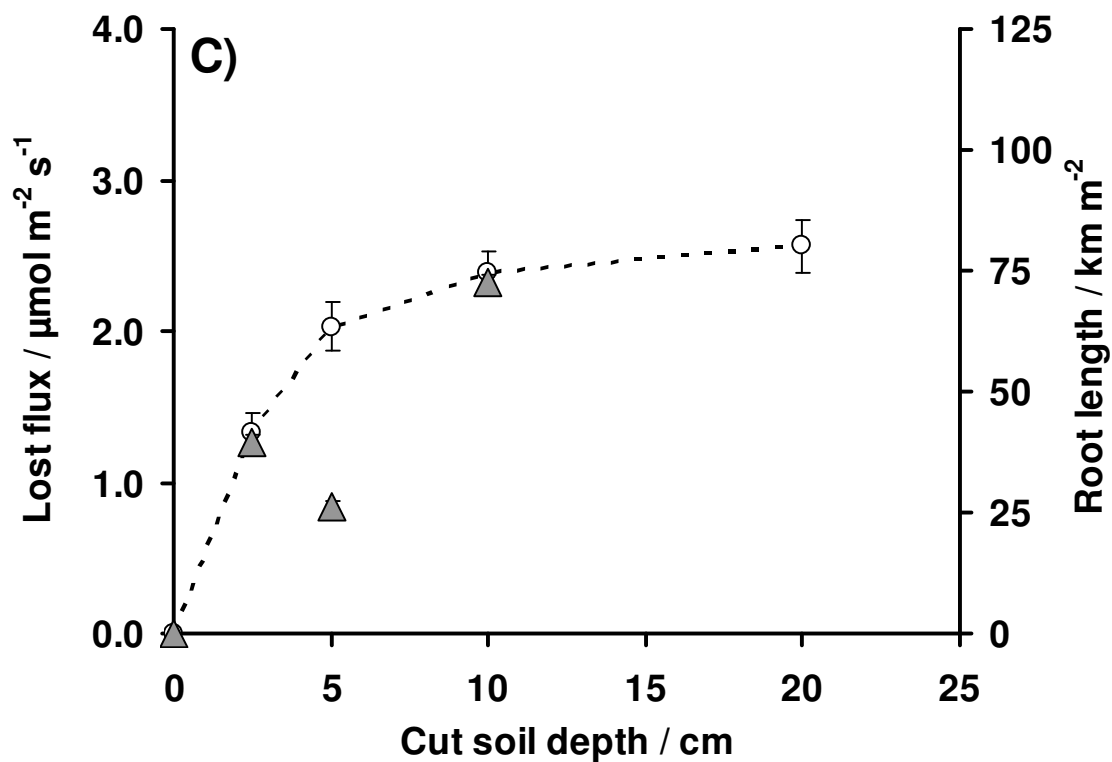


Table 1 Mean root length (RL) and mean root diameter (mm) per soil segment layer (depth increments) and the cumulative mean root length CRL (km m^{-2}); obtained from soil coring at the peatland, forest and grassland site; number of core replicates were $n = 3, 6$ and 4 , respectively. Note: the average diameter of the peatland roots in the top two layers was smaller (i.e. 0.41 and 0.38 mm, respectively) when including the large amount of very fine roots (average diameter of 0.33 mm); $n = 3$ (peatland) and 6 (forest). One-way ANOVA with an LSD *post-hoc* test showed that for the peatland all dependent variables were significantly different between depths at least at the $**P < 0.01$ level (except for the CRL, where only the $0 - 5$ cm layer differed from the others at the $*P < 0.05$ level); for the forest site the two upper layers of the root length density RLD (cm cm^{-3}) and all the CRL (km m^{-2}) data were significantly different from all others at least at the $**P < 0.01$ level; for the grassland site nearly all properties showed significant differences at at least at the $**P < 0.01$ level between them, except for RLD (cm cm^{-3}) and CRL, which were significant only at the $*P < 0.05$ level, and total RL, RLD (cm cm^{-3}) and CRL for the two deepest depths, and all diameter classes showed no significant differences.

Table 2 Comparison of mean soil respiration fluxes according to sampling times (e.g. 12:00 - 14:00 or 18:00 – 00:00 hours compared with a 24-hour period) and collar depth (cm) during consecutive days (peatland: 10 – 19 October 2006; forest: wet (soil moisture 20-29%, v/v) 14 – 18 and dry 20 – 29 June (soil moisture 11-20%, v/v) 2006; grassland: 13 – 30 June 2008). Note the SE values are based on averaging hourly mean values over several days (n = 9; n = 5, 10; n = 10 for peatland, forest (wet, dry) and grassland, respectively). Significant differences are based on comparing means in a two-way ANOVA with an LSD *post-hoc* test (different letters indicate significant differences): peatland 0 cm **P* = 0.068; forest *wet* 5 cm ****P* < 0.001; 10 cm **P* = 0.021; grassland 0 cm **P* < 0.05; 2.5 cm **P* = 0.05; 5 cm ***P* = 0.01; 10 cm ***P* = 0.01; n = 9 (peatland), n= 5 and 9 (forest, wet and dry, respectively) and n = 10 (grassland).

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